

# CHEMISTRY

## A European Journal

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### Accepted Article

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This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

**To be cited as:** *Chem. Eur. J.* 10.1002/chem.201705902

**Link to VoR:** <http://dx.doi.org/10.1002/chem.201705902>

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# A gold(III) pincer ligand scaffold for the synthesis of binuclear and bioconjugated complexes: synthesis and anticancer potential

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**Abstract:** Cyclometallated ( $C^N^N C$ )Au(III) complexes bearing functionalized N-heterocyclic carbene (NHC) ligands provide a high-yielding, modular route to bioconjugated and binuclear complexes. This methodology has been applied to the synthesis of bioconjugated complexes presenting biotin and 17 $\alpha$ -ethynylestradiol vectors, as well as to the synthesis of bimetallic Au(III)/Au(I) complexes. The *in vitro* antiproliferative activities of these compounds against various cancer cells lines depend on the linker length, with the longer linker being the most potent. The estradiol conjugate **AuC<sub>6</sub>Estra** proved to be more toxic against the estrogen receptor positive (ER+) cancer cells than against the ER- cancer cells and non-cancer cells. The bimetallic complex **AuC<sub>6</sub>Au** was more selective for breast cancer cells with respect to a healthy cell standard than the monometallic complex **AuNHC**. The metal uptake study on cells expressing or not biotin and estrogen receptors revealed an improved and targeted delivery of gold for both the bioconjugated complexes **AuC<sub>6</sub>Biot** and **AuC<sub>6</sub>Estra** compared to the non-vectorised analogue **AuNHC**. The investigations of the interaction of the bioconjugates and bimetallic complexes with human telomeric G-quadruplex DNA using FRET-melting techniques revealed a reduced ability to stabilize this DNA structure with respect to the non-vectorised analogue **AuNHC**.

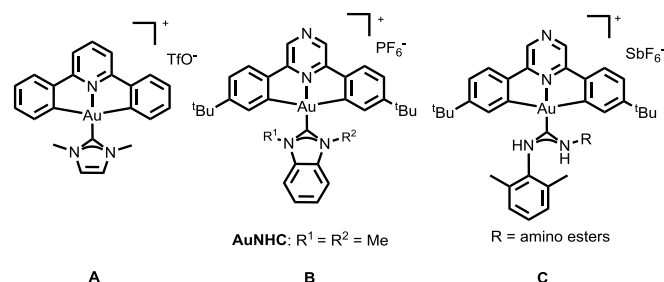
## Introduction

Cyclometallated Au(III) complexes have attracted significant interest in the last decade for a large palette of applications including fundamental gold chemistry investigations,<sup>[1]</sup> photoemissive materials<sup>[2]</sup> and biological applications.<sup>[3]</sup> Bis-cyclometallated ( $C^N^N C$ )Au(NHC) complexes have appeared as particularly promising anticancer agents (see Figure 1 for examples).<sup>[4]</sup>

Those complexes have been indicated to enhance topoisomerase 1 induced DNA cleavage,<sup>[4a]</sup> to downregulate protein synthesis pathways<sup>[4c]</sup> and to stabilize G-quadruplex and i-motif structures in DNA (Figure 1, type **A** and **B**).<sup>[5]</sup> We have demonstrated that a pyrazine-based [ $(C^N^N C)Au(NHC)]^+$  complex (**AuNHC** in

Figure 1), which presents low- to sub-micromolar IC<sub>50</sub> values on various cancer cell lines, was highly stable toward reduction and substitution in physiological environments, even in the presence of glutathione. However, its low selectivity for cancer cells with respect to healthy ones remains a challenge.<sup>[5]</sup> Modifying the NHC ligand in structure **B** (Figure 1), e.g. by decorating substituents R<sup>1</sup>, R<sup>2</sup> with suitable vectors, seemed a promising strategy to improve the selectivity for cancer cells and to decrease potential side effects. Conjugation to biological vectors and use of polynuclear compounds have been shown to lead to increases in the selectivity and toxicity of anticancer drugs.<sup>[6,7,8,9]</sup> To this end, we recently investigated the synthesis of amino ester-decorated ( $C^N^N C$ )Au(III) acyclic carbene complexes (Figure 1, type **C**), made by the nucleophilic attack by the corresponding amino esters on cationic ( $C^N^N C$ )Au(III) isocyanide complexes. However, although that methodology was successful when using amino esters, the requirement to work under perfectly anhydrous conditions precluded the use of more elaborate biomolecules. Moreover, those acyclic carbene complexes were found to be much less stable toward GSH than cyclic NHC-based complexes.<sup>[10]</sup>

Imidazole- and benzimidazole-type NHC ligands bearing pentafluorophenyl ester moieties have already been successfully used for grafting organometallic Ru(II) and Au(I) complexes onto peptides,<sup>[11]</sup> aptamers<sup>[12]</sup> and for the synthesis of bimetallic complexes.<sup>[13]</sup> The synthesis of bioconjugated metal complexes often requires considerable synthetic efforts in order to achieve modularity in terms of potential vectors and also in terms of linkers between the pharmacophore and the vector.<sup>[14]</sup> Thus, using an NHC ligand featuring a pentafluorophenyl ester group to build a single scaffold for the bioconjugation of different vectors or metal-based fragment onto a ( $C^N^N C$ )Au(III) core could lead to large library of biologically relevant compounds in only few synthetic steps.



**Figure 1:** Examples of [ $(C^N^N C)Au(NHC)]^+$  complexes investigated for anticancer purposes.<sup>[4a,5,10]</sup>

Here we report a high-yield three-step procedure for the synthesis of bioconjugated and bimetallic complexes using a common [ $(C^N^N C)Au(NHC)]^+$  precursor complex of type **B** bearing a pentafluorophenyl ester group on the NHC ligand. We used this precursor for the synthesis of biotin, 17 $\alpha$ -ethynylestradiol and

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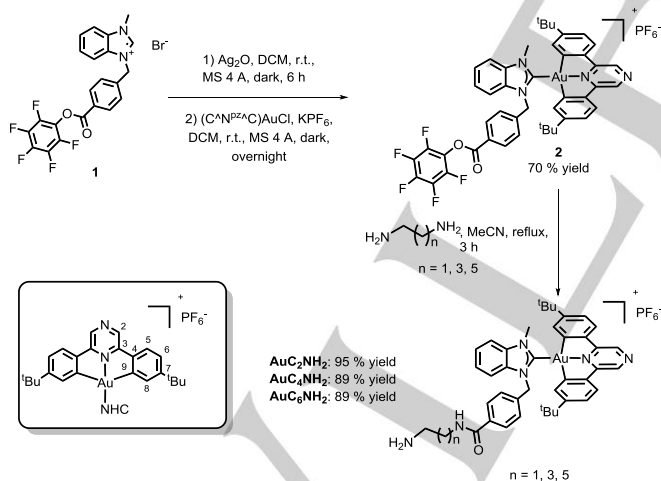
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benzimidazole-based (NHC)AuCl conjugates connected by linkers of variable length. The different conjugates and bimetallic complexes have been investigated *in vitro* for their antiproliferative properties on human cancer cell lines.

## Results and Discussion

**Synthesis and characterization.** The synthetic route is shown in Scheme 1. Complex **2** was obtained in 70% yield by the classical transmetalation method, starting by reacting the benzimidazolium salt with silver oxide to afford the silver-NHC complex, followed by NHC transfer to  $(C^{\wedge}N^{PZ^{\wedge}}C)AuCl$ . The reaction of **2** with diaminoalkanes of different chain lengths (i. e. ethylenediamine, 1,4-diaminobutane, and 1,6-diaminohexane) in refluxing acetonitrile gave high isolated yields of the corresponding  $[(C^{\wedge}N^{PZ^{\wedge}}C)Au(NHC)]^{+}$  derivatives with pendent amine substituents, **AuC<sub>n</sub>NH<sub>2</sub>** (*n* = 2, 4, 6) (Scheme 1). The  $^{19}F\{^1H\}$  NMR spectra confirmed the quantitative conversion of the pentafluorophenol group. The  $^1H$  NMR spectra showed the loss of symmetry of the various diaminoalkyl linkers associated with the lower frequency shift of the signals of the benzylic protons due to the increase of electron density upon substitution of the pentafluorophenol ester by an amine. In the **AuC<sub>n</sub>NH<sub>2</sub>** complexes, the  $^{13}C\{^1H\}$  NMR C=O signals are shifted from  $\delta$  161 ppm in **2** to 165–166 ppm in the amide products, depending of the length of the alkyl chain. Amide formation was further corroborated by the shift of the  $\nu(C=O)$  band in the IR spectra from 1760  $cm^{-1}$  to 1650  $cm^{-1}$ . All these features are in good agreement with reported reactions.<sup>[13]</sup>



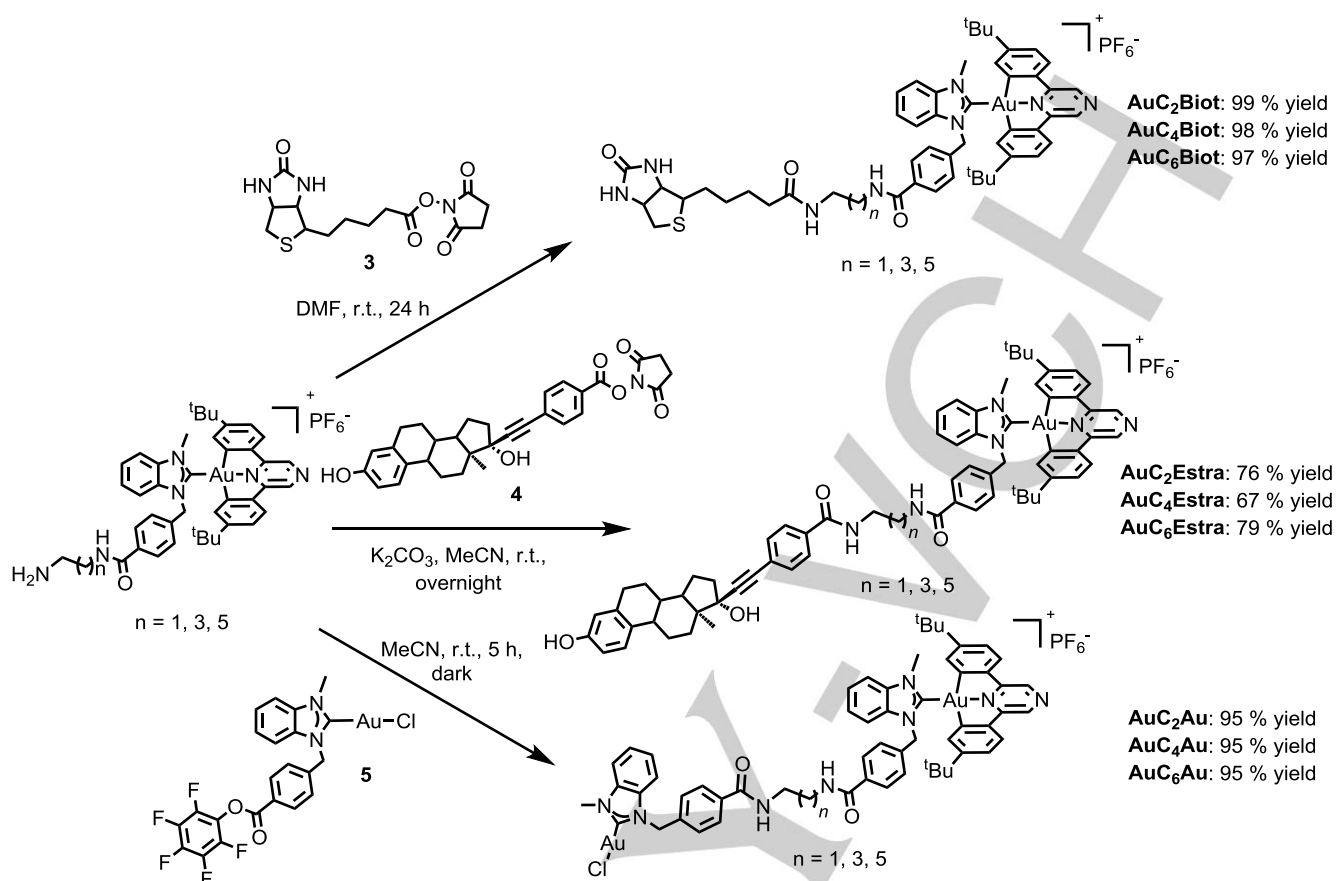
**Scheme 1:** Synthesis of the complexes **AuC<sub>n</sub>NH<sub>2</sub>** (*n* = 2, 4, 6).

The **AuC<sub>n</sub>NH<sub>2</sub>** complexes were converted to bioconjugated and binuclear complexes as shown in Scheme 2. Biotin has already been used for the targeting of taxol<sup>[15]</sup> and metal complexes.<sup>[4c,16]</sup> To obtain gold(III) biotin conjugates, **AuC<sub>n</sub>NH<sub>2</sub>** complexes were reacted

with the biotin-*N*-hydroxysuccinimide (biotin-NHS) precursor **3** at room temperature for 24 hours. Precipitation with Et<sub>2</sub>O afforded the Au(III)/biotin conjugates **AuC<sub>n</sub>Biot** (*n* = 2, 4, 6) in almost quantitative isolated yields. The success of the reactions was confirmed by  $^1H$  NMR spectroscopy which showed a 1/1 ratio between the signals of the biotin and  $[(C^{\wedge}N^{PZ^{\wedge}}C)Au(NHC)]^{+}$  fragment, coupled with the disappearance of the NHS signal and the appearance of a signal of a second amide proton. The IR spectra of the **AuC<sub>n</sub>Biot** complexes displayed two  $\nu(C=O)$  bands at 1704  $cm^{-1}$  and 1646  $cm^{-1}$  corresponding, respectively, to the urea and amide functional groups. The formation of the expected conjugates was further confirmed by high resolution mass spectrometry, which showed in each case the molecular monocation and the dicationic adducts with sodium or potassium.

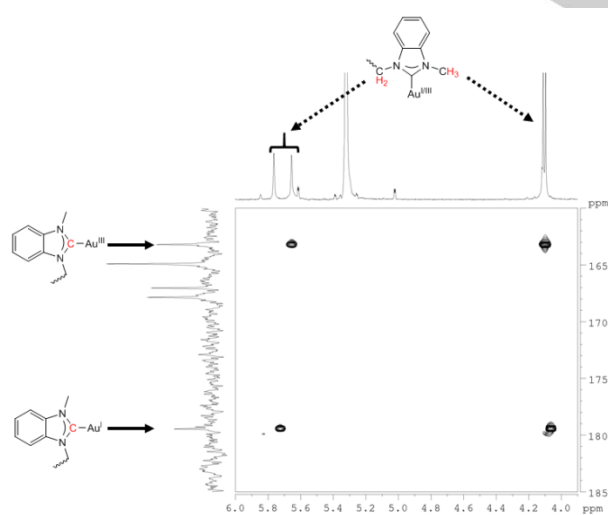
Estradiol/metal complex conjugates have been used to target estrogen receptor positive (ER+) cells.<sup>[14a,17]</sup> By reacting the **AuC<sub>n</sub>NH<sub>2</sub>** complexes with the 17 $\alpha$ -ethynylestradiol-NHS precursor **4** at room temperature in acetonitrile in presence of a base, the Au(III)/estradiol conjugates **AuC<sub>n</sub>Estra** (*n* = 2, 4, 6) were obtained in good isolated yield (Scheme 2). Those yields were more than 25% higher than the previously reported coupling methods between metal-NHC complexes and estradiol derivatives using ruthenium-catalyzed alkyne-azide cycloaddition.<sup>[17a]</sup> The formation of the bioconjugated species was indicated in the  $^1H$  NMR spectrum by the 1/1 ratio of the signals for the  $[(C^{\wedge}N^{PZ^{\wedge}}C)Au(NHC)]^{+}$  core and the estradiol vector, together with the disappearance of the signal of the NHS group. IR spectroscopy showed in all cases only one signal for the carbonyl function, as previously seen for the biotin conjugates. The formation of the expected conjugates was also confirmed by high resolution mass spectrometry, which in each case showed the molecular cation.

Few studies have reported mixed valence Au(I)/Au(III) complexes and, to the best of our knowledge, never with organogold fragments.<sup>[18]</sup> By reacting the **AuC<sub>n</sub>NH<sub>2</sub>** complexes with the Au(I)-NHC precursor **5** in acetonitrile at room temperature, the mixed valence binuclear complexes **AuC<sub>n</sub>Au** (*n* = 2, 4, 6) were obtained with very high isolated yields (Scheme 2). The  $^1H$  NMR spectra of the binuclear complexes displayed a 1/1 ratio between the signals of the Au(III) and Au(I) fragments, a signal for a second amide proton and the quasi-equivalence of the two CH<sub>2</sub> groups in a position with respect to the amides. The  $^{13}C\{^1H\}$  NMR spectra of the **AuC<sub>n</sub>Au** complexes showed two different carbene-C signals at  $\delta$  179 and 163 ppm for the Au(I)-NHC and Au(III)-NHC fragments, respectively. These signals were identified using  $^1H$ - $^{13}C$  2D-NMR spectroscopy which showed the correlation between the methyl and methylene side-chain of each carbene with the corresponding carbene-C signals (Figure 2). The formation of the expected bimetallic complexes was also confirmed by high resolution mass spectrometry which showed in each case the molecular monocation and the dication resulting from the loss of the chloride ligand.

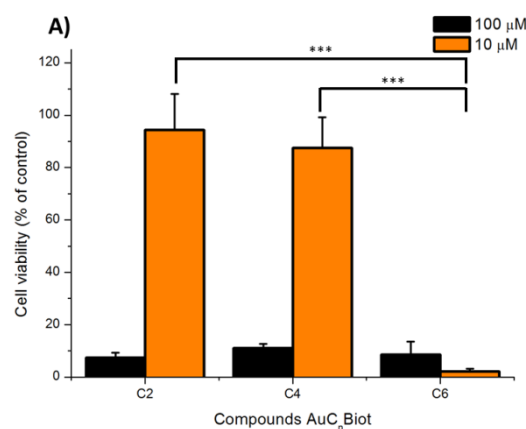


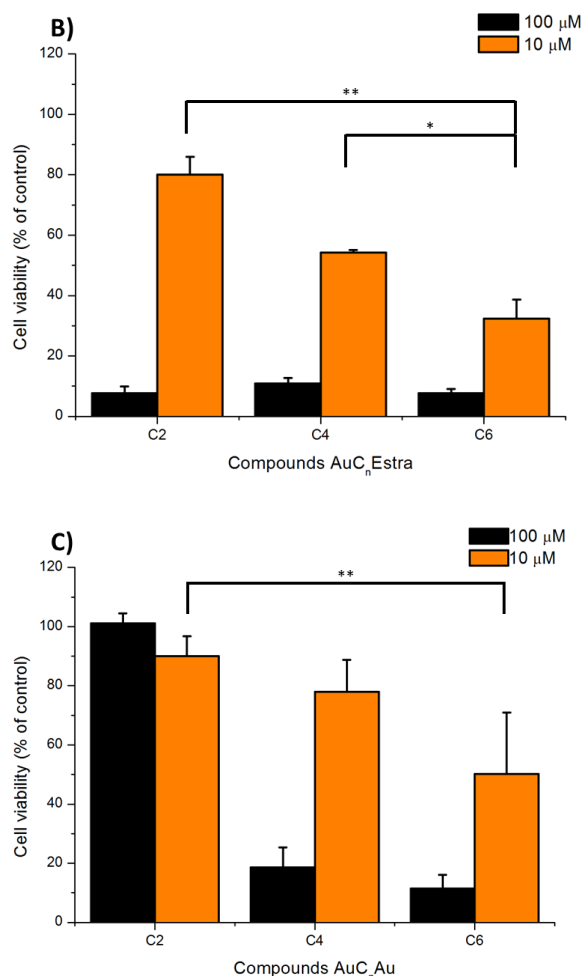
**Scheme 2:** Synthesis of the bioconjugated complexes **AuC<sub>n</sub>Biot** and **AuC<sub>n</sub>Estra** ( $n = 2, 4, 6$ ) and the binuclear complexes **AuC<sub>n</sub>Au** ( $n = 2, 4, 6$ ).

**Evaluation of antiproliferative activity.** All conjugated and binuclear compounds were screened at concentrations of 100  $\mu\text{M}$  and 10  $\mu\text{M}$  against MCF-7 cells, which are known to express both biotin and estrogen receptors.<sup>[14a,15]</sup> Cell viability was measured after 72 hours of incubation by the classical MTS assay (see Experimental Section). The results are depicted in Figure 3.



**Figure 2:** HMBC  $^1\text{H}$ - $^{13}\text{C}$  2D correlation spectra of **AuC<sub>2</sub>Au** showing the two different NHC-Au<sup>III</sup> moieties. Spectra recorded in  $\text{CD}_2\text{Cl}_2$  at 298 K.





**Figure 3:** Inhibition of MCF-7 cells proliferation by complexes A) **AuC<sub>n</sub>Biot** ( $n = 2, 4, 6$ ); B) **AuC<sub>n</sub>Estra** ( $n = 2, 4, 6$ ); C) **AuC<sub>n</sub>Au** ( $n = 2, 4, 6$ ). Data represent the average  $\pm$  standard deviation of three experiments. The significance of the results was analysed by t-test. \* $p$  value  $< 0.05$ ; \*\* $p$  value  $< 0.01$ ; \*\*\* $p$  value  $< 0.001$ .

The data show a correlation between the length of the alkyl linker and the toxicity of the respective systems, with the C<sub>6</sub> complexes being significantly more toxic than the C<sub>2</sub> and C<sub>4</sub> congeners, except for the **AuC<sub>n</sub>Au** complexes where the difference between the C<sub>6</sub> and

C<sub>4</sub> compounds is not significant. For that reason **AuC<sub>6</sub>Biot**, **AuC<sub>6</sub>Estra** and **AuC<sub>6</sub>Au** were selected for further cytotoxic investigations. The complexes **AuC<sub>6</sub>Biot** and **AuC<sub>6</sub>Estra** were tested on panels of cells expressing or not expressing biotin receptors (BR) and estrogen receptors (ER), respectively. Tests on normal healthy fibroblasts were included for comparison. Results are reported in Table 1.

The antiproliferative profile of **AuC<sub>6</sub>Biot** does not seem to depend on the expression of the biotin receptors (Table 1). Indeed, **AuC<sub>6</sub>Biot** appeared only marginally more potent against some of the BR+ cell lines (MCF-7 and MDA-MB-231) than against BR- cell lines (HCT-116 and HEK-293) or the healthy fibroblasts (MRC5) ( $IC_{50} = 8.9 \pm 0.7$ ;  $9.6 \pm 1.6$ ;  $11.2 \pm 0.9$ ;  $10.3 \pm 0.4$  and  $11 \pm 5 \mu M$  against MCF-7, MDA-MB-231, HCT-116, HEK-293 and MRC-5 cells respectively). The introduction of the polar biotin substituent into the NHC framework greatly reduced the cytotoxicity, e.g. against MCF-7 by a factor of  $\sim 16$  compared to **AuNHC**. Moreover **AuC<sub>6</sub>Biot** appeared completely non-toxic against A549 cells although these are reported to largely express biotin receptors.<sup>[15]</sup>

A different situation was encountered in the case of **AuC<sub>6</sub>Estra**. This complex was tested against MCF-7 (ER+), MDA-MB-231 (ER-) and MRC-5 (healthy fibroblasts) cells (Table 1). The antiproliferative effect of **AuC<sub>6</sub>Estra** against the ER+ cells was slightly higher than against the ER- and non-cancer cells. This may suggest a possible targeting effect of the 17 $\alpha$ -ethynylestradiol vector.

Interestingly, **AuC<sub>6</sub>Au** presented a specificity for the breast cancer cell lines MCF-7 and MDA-MB-231 ( $IC_{50} = 13.7 \pm 2.2$  and  $9.2 \pm 2.9 \mu M$ , respectively) compared to the other cancer cells A549 (lung) and HCT116 (colon) ( $IC_{50} > 100 \mu M$  in both cases) (Table 1). **AuC<sub>6</sub>Au** showed the same ratio between its  $IC_{50}$  values against MCF-7 cells and healthy fibroblasts ( $IC_{50} = 38.1 \pm 5.7 \mu M$ ) as **AuNHC**. Moreover, the  $IC_{50}$  value of **AuC<sub>6</sub>Au** was four times lower against MDA-MB-231 cells than against MRC-5 cells. This contrasts with the unsubstituted Au(III) complex **AuNHC** which proved four times more toxic against healthy fibroblasts than against MDA-MB-231 cells. The results demonstrate a promising increase in anti-cancer selectivity as a result of NHC modification.

**Table 1:** Comparison of the effects of **AuC<sub>6</sub>Biot**, **AuC<sub>6</sub>Estra**, **AuC<sub>6</sub>Au** and **AuNHC** on cell viability of a panel of human cancer cell lines, of human embryonic kidney HEK-293 cells and of healthy fibroblast MRC-5 cells after 72 h incubation.

Cell line	receptor expression <sup>[14a,15]</sup>	$IC_{50}$ ( $\mu M$ ) <sup>a</sup>			
		<b>AuC<sub>6</sub>Biot</b>	<b>AuC<sub>6</sub>Estra</b>	<b>AuC<sub>6</sub>Au</b>	<b>AuNHC</b>
<b>MCF-7</b>	ER+/BR+	$8.9 \pm 0.7$	$5.9 \pm 0.4$	$13.7 \pm 2.2$	$0.56 \pm 0.02^5$
<b>MDA-MB-231</b>	ER-/BR+	$9.6 \pm 1.6$	$9.3 \pm 1.2$	$9.2 \pm 2.9$	$5.7 \pm 0.4^{10}$
<b>A549</b>	BR+	$> 100$	n. d.	$> 100$	$7.8 \pm 1.3^5$
<b>HCT-116</b>	BR-	$11.2 \pm 0.9$	n. d.	$> 100$	$11.2 \pm 1.5^{10}$
<b>HEK-293</b>	BR-	$10.3 \pm 0.4$	n. d.	n. d.	$5.2 \pm 0.3$
<b>MRC-5</b>	n. d.	$11 \pm 5$	$9.1 \pm 0.6$	$38.1 \pm 5.7$	$1.4 \pm 0.4^5$

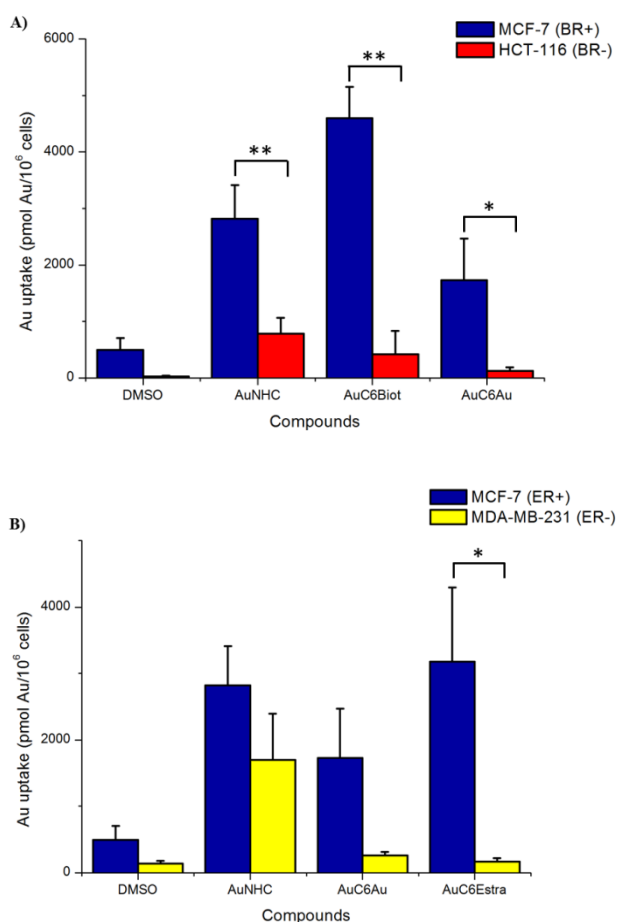
<sup>a</sup> Data represent the average  $\pm$  standard deviation of three experiments. n. d.: not determined.



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**Cellular gold uptake.** Drug uptake and intracellular metal accumulation has been demonstrated to be one of the main factors limiting drug efficacy.<sup>[19]</sup> The role of biovectors on the uptake of metal-based drugs can be quantified using inductively coupled plasma-mass spectrometry techniques (ICP-MS); this method has already been used in order to assess the role of biovectors on the uptake of metal-based drugs.<sup>[20]</sup> We investigated the impact of the vectors and the presence of a second gold centre on the accumulation of our complexes by measuring the gold uptake in MCF-7 cells (ER+ and BR+), HCT-116 (BR-) and MDA-MD-231 (ER-). The results are depicted in Figure 4.

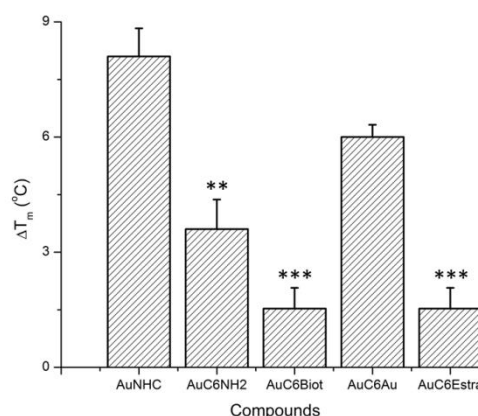


**Figure 4:** Impact of the vector and second gold center on the cell uptake by A) MCF-7 (BR+) and HCT-116 (BR-) cells; B) MCF-7 (ER+) and MDA-MB-231 (ER-) cells after 6 hours of treatment with 10  $\mu$ M of compounds in DMSO; data represent the average and standard deviations of three experiments. The significance of the results was analyzed by t-test. \* $p$  value < 0.05; \*\* $p$  value < 0.01.

Interestingly, both the biotin and 17 $\alpha$ -ethynylestradiol vector improved the gold uptake with respect to the non-conjugated complex **AuNHC**. Moreover, for both **AuC6Biot** and **AuC6Estra** the cells expressing the corresponding receptors (BR for **AuC6Biot** and ER for **AuC6Estra**) presented a significantly higher intracellular gold content than the cells not expressing the receptors. This suggests the potential of using such vectors to effectively and selectively

deliver metal-based compounds to selected cancer cells. However, despite the enhanced intracellular accumulation, both **AuC6Biot** and **AuC6Estra** appeared less cytotoxic than **AuNHC**, which suggests that cell uptake might not be the limiting factor for those compounds. In contrast, despite having two gold atoms per molecule, **AuC6Au** showed a reduced intracellular concentration of gold in all cancer cells with respect to the mono-gold complex **AuNHC**. This is in line with the reduced antiproliferative activity of **AuC6Au**. Moreover, **AuC6Au** presented a significantly reduced gold uptake in HCT-116 cells where it demonstrated no antiproliferative activity.

**Interaction with DNA G-quadruplex.** G-quadruplexes are secondary structures of DNA formed by stacking of tetrads of guanines residues linked via Hoogsteen hydrogen-bonding and stabilized by the presence of typically monovalent cations in the center of the tetrad.<sup>[21]</sup> We recently reported on the ability of **AuNHC** to stabilize the G-quadruplex structure of DNA as a possible intracellular target for these class of compounds.<sup>[5]</sup> To highlight the impact of the vectors and the second gold centre on the interaction of the [(C<sup>N</sup>N<sup>BZ</sup>^C)Au(NHC)]<sup>+</sup> core with potential targets, **AuC6Biot**, **AuC6Au**, **AuC6Estra** and **AuC6NH<sub>2</sub>** were screened against the human telomeric G-quadruplex sequence (5'-GGG-TTA-GGG-TTA-GGG-TTA-GGG-3', hTeloG) at pH 7.2 by a FRET-based DNA melting assay (see Experimental). The results are presented in Figure 5.



**Figure 5:** Stabilization of human telomeric DNA G-quadruplex sequence (hTeloG, 0.2  $\mu$ M) in 10 mM sodium cacodylate, 100 mM NaCl at pH 7.2 by complexes **AuNHC**, **AuC6NH<sub>2</sub>**, **AuC6Biot**, **AuC6Au** and **AuC6Estra** at 1  $\mu$ M measured by FRET-DNA melting assay; data represent the average and standard deviations of three experiments. The significance of the results was analyzed by t-test. \*\* $p$  value < 0.01; \*\*\* $p$  value < 0.001.

Compared to the parent compound, **AuNHC**, we could observe a reduced interaction with hTeloG due to the presence of the long 1,6-diaminohexane linker ( $\Delta T_m = 3.6 \pm 0.8^\circ\text{C}$  for **AuC6NH<sub>2</sub>**). Furthermore the presence of the two vectors reduced even more the interaction with hTeloG with respect to **AuC6NH<sub>2</sub>** ( $\Delta T_m = 1.6 \pm 0.6$ ;  $1.4 \pm 0.6^\circ\text{C}$  for **AuC6Biot** and **AuC6Estra** respectively). Interestingly, although NHC-Au(I)-Cl compounds have been reported not to stabilize G-quadruplex structures,<sup>[22]</sup> the presence of the second gold centre enhanced the interaction with hTeloG with respect to **AuC6NH<sub>2</sub>** ( $\Delta T_m = 6.0 \pm 0.3^\circ\text{C}$  for **AuC6Au**) and was close to the strength of the

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interaction between **AuNHC** and hTeloG ( $\Delta T_m = 8.1 \pm 0.7^\circ\text{C}$  for **AuNHC**). Overall, these data suggest, at least for the example of the G-quadruplex DNA structures, that whilst the presence of either the biotin or the 17 $\alpha$ -ethynylestradiol improved the uptake of  $[(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{C})\text{Au}(\text{NHC})]^+$ , at the same time they decreased the ability of the  $[(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{C})\text{Au}(\text{NHC})]^+$  core to interact with a possible intracellular target. This inhibition of interaction may be correlated with the observed decreased antiproliferative activity. On the contrary, having a second gold centre does not dramatically reduce the interaction with the target but decreased the cell uptake, resulting also in a generally reduced antiproliferative activity.

## Conclusions

In summary, the synthesis of a library of bioconjugated and binuclear  $[(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{C})\text{Au}(\text{NHC})]^+$  complexes has been achieved in high yields employing a modular approach from a common Au(III) scaffold. This has allowed the easy screening of several molecular design parameters such as the linker length, the biological vector used and the number of metal centres. While the biotin and 17 $\alpha$ -ethynylestradiol vectors improved and targeted the gold uptake to cells expressing the relevant receptors, they did not increase the antiproliferative activities, likely due to a reduction in the interaction of these sterically more hindered complexes with possible intracellular targets. By contrast, the reduced activity of the bimetallic complex **AuC<sub>6</sub>Au** was mainly driven by a reduced intracellular accumulation. On the other hand, **AuC<sub>6</sub>Au** was up to 4.1 times more toxic towards MDA-MB-231 cancer cells than towards healthy cells. Further studies are ongoing to increase the activity of the complexes by introducing "self-immolative" linkers<sup>[23]</sup> in order to enable the separation of the biovector from the  $[(\text{C}^{\text{N}}\text{N}^{\text{C}})\text{Au}(\text{NHC})]^+$  pharmacophore once inside the cancer cells.

## Experimental Section

**General Remarks.** Anhydrous solvents were freshly distilled from appropriate drying agents.  $^1\text{H}$  and  $^{13}\text{C}\{^1\text{H}\}$  spectra were recorded using a Bruker Avance DPX-300 spectrometer.  $^1\text{H}$  NMR spectra (300.13 MHz) were referenced to the residual protons of the deuterated solvent used.  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra (75.47 MHz) were referenced internally to the D-coupled  $^{13}\text{C}$  resonances of the NMR solvent. Elemental analyses were carried out at London Metropolitan University. Compounds **1**, **3**, **4**, **5** and the gold precursor  $(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{C})\text{AuCl}$  were synthesized following reported procedures.<sup>[2c,12,24,25]</sup>

## Synthesis and characterization

$[(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{N})\text{Au}(1\text{-methyl-3-(4-carboxypentafluorophenyl)benzylbenzimidazol-2-ylidene})]\text{PF}_6$  (**2**)

A mixture of **1** (45 mg, 0.087 mmol) and **Ag<sub>2</sub>O** (20 mg, 0.087 mmol) was stirred in distilled dichloromethane (20 mL) at room temperature in the dark for 6 h.  $(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{C})\text{AuCl}$  (50 mg, 0.087 mmol) and **KPF<sub>6</sub>** (49 mg, 0.261 mmol) were added to the mixture and the reaction was stirred overnight at room temperature in the dark. The obtained yellow solution was filtered through Celite and the solvent was removed *in vacuo* to give a yellow film. Upon washing with light petroleum a yellow powder was formed which was collected and

dried to afford the pure product (68 mg, 0.061 mmol, 70 % yield). Anal. Calcd for  $\text{C}_{46}\text{H}_{39}\text{N}_4\text{O}_2\text{F}_5\text{AuPF}_6$  (1116.8): C, 49.47; H, 3.52; N, 5.02. Found: C, 49.51; H, 3.37; N, 5.20. IR ( $\text{cm}^{-1}$ ):  $\nu(\text{C}=\text{O})$  1760.  $^1\text{H}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 300.13 MHz):  $\delta$  8.97 (s, 2 H,  $\text{H}^2$ ), 7.86 (d + m,  $^3J_{\text{H-H}} = 9.0$  Hz, 4 H,  $\text{H}^{12} + \text{H}^{15} + \text{H}^{19}$ ), 7.75 (d + m,  $^3J_{\text{H-H}} = 9.0$  Hz, 4 H,  $\text{H}^5 + \text{H}^{13} + \text{H}^{14}$ ), 7.43 (dd,  $^3J_{\text{H-H}} = 9.0$  Hz,  $^4J_{\text{H-H}} = 3.0$  Hz, 2 H,  $\text{H}^6$ ), 7.37 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 2 H,  $\text{H}^{18}$ ), 6.88 (d,  $^4J_{\text{H-H}} = 3.0$  Hz, 2 H,  $\text{H}^8$ ), 5.75 (s, 2 H, N-CH<sub>2</sub>), 4.15 (s, 3 H, N-CH<sub>3</sub>), 1.18 (s, 18 H, <sup>t</sup>Bu).  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 75.48 MHz, 293 K):  $\delta$  164.9 (s,  $\text{C}^9$ ), 163.3 (s,  $\text{C}_{\text{carbene}}$ ), 161.9 (s,  $\text{C}=\text{O}$ ), 158.4 (s,  $\text{C}^7$ ), 157.2 (s,  $\text{C}^4$ ), 144.7 (s,  $\text{C}^3$ ), 141.0 (s,  $\text{C}^{20}$ ), 140.0 (s,  $\text{C}^2$ ), 134.8 (s,  $\text{C}^{16}$ ), 134.3 (s,  $\text{C}^{11}$ ), 133.3 (s,  $\text{C}^8$ ), 131.9 (s,  $\text{C}^{19}$ ), 129.0 (s,  $\text{C}^5$ ), 128.1 (s,  $\text{C}^{17}$ ), 127.5 (s,  $\text{C}^{18}$ ), 127.4 (s,  $\text{C}^{13/14}$ ), 127.3 (s,  $\text{C}^{13/14}$ ), 126.2 (s,  $\text{C}^6$ ), 113.0 (s,  $\text{C}^{12/15}$ ), 112.8 (s,  $\text{C}^{12/15}$ ), 52.3 (s, CH<sub>2</sub>-N), 36.8 (s, N-CH<sub>3</sub>), 35.7 (s,  $\text{C}(\text{CH}_3)_3$ ), 31.1 (s,  $\text{C}(\text{CH}_3)_3$ ).  $^{19}\text{F}\{^1\text{H}\}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 282.38 MHz, 293 K)  $\delta$  -73.2 (d,  $^1J_{\text{P-F}} = 711$  Hz, 6 F, PF<sub>6</sub>), -153.1 (d,  $^3J_{\text{F-F}} = 16.9$  Hz, 2 F, CF<sub>ortho</sub>), -158.2 (t,  $^3J_{\text{F-F}} = 21.2$  Hz, 1 F, CF<sub>para</sub>), -162.8 (dd,  $^3J_{\text{F-F}} = 19.7$  Hz and 22.6 Hz, 2 F, CF<sub>meta</sub>).

$[(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{N})\text{Au}(1\text{-methyl-3-(4-(2-aminoethyl)carboxamid)benzylbenzimidazol-2-ylidene})]\text{PF}_6$  (**AuC<sub>2</sub>NH<sub>2</sub>**)

A mixture of **2** (250 mg, 0.22 mmol) and ethylenediamine (0.3 mL, 4.4 mmol) was stirred in distilled acetonitrile (20 mL) at 80 °C for 3 h. After the solution was cooled to room temperature, acetonitrile was removed under vacuum, leaving an orange oily solid which was dissolved into dichloromethane. The organic phase was washed twice with a saturated aqueous NaHCO<sub>3</sub> solution and twice with water. After drying over Na<sub>2</sub>SO<sub>4</sub>, the solvent was partially evaporated. Upon addition of light petroleum an orange precipitate was formed which was collected and dried to afford the pure product as an orange powder (208 mg, 0.21 mmol, 95 % yield). Anal. Calcd for  $\text{C}_{42}\text{H}_{46}\text{N}_6\text{OAU}\text{PF}_6$  (992.8): C, 50.81; H, 4.67; N, 8.47. Found: C, 50.69; H, 4.43; N, 8.56. IR ( $\text{cm}^{-1}$ ):  $\nu(\text{N-H})$  3441,  $\nu(\text{N-H})$  3299,  $\nu(\text{C}=\text{O})$  1650.  $^1\text{H}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 300.13 MHz):  $\delta$  8.94 (s, 2 H,  $\text{H}^2$ ), 7.83-7.70 (d + m,  $^3J_{\text{H-H}} = 9.0$  Hz, 6 H,  $\text{H}^5 + \text{H}^{13-16}$ ), 7.52 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 2 H,  $\text{H}^{19}$ ), 7.40 (dd,  $^3J_{\text{H-H}} = 9.0$  Hz,  $^4J_{\text{H-H}} = 3.0$  Hz, 2 H,  $\text{H}^6$ ), 7.20 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 3 H,  $\text{H}^{18} + \text{C}(\text{O})\text{NH}$ ), 6.89 (d,  $^4J_{\text{H-H}} = 3.0$  Hz, 2 H,  $\text{H}^8$ ), 5.67 (s, 2 H, N-CH<sub>2</sub>), 4.13 (s, 3 H, N-CH<sub>3</sub>), 3.36 (m, 2 H,  $\text{C}(\text{O})\text{NH-CH}_2$ ), 2.86 (t,  $^3J_{\text{H-H}} = 6.0$  Hz, CH<sub>2</sub>-NH<sub>2</sub>), 2.70 (broad s, 2 H, NH<sub>2</sub>), 1.18 (s, 18 H, <sup>t</sup>Bu).  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 75.48 MHz, 293 K):  $\delta$  166.5 (s,  $\text{C}=\text{O}$ ), 165.2 (s,  $\text{C}^9$ ), 163.2 (s,  $\text{C}_{\text{carbene}}$ ), 158.3 (s,  $\text{C}^7$ ), 157.2 (s,  $\text{C}^4$ ), 144.8 (s,  $\text{C}^3$ ), 140.0 (s,  $\text{C}^2$ ), 136.9 (s,  $\text{C}^{20}$ ), 135.6 (s,  $\text{C}^{17}$ ), 134.8 (s,  $\text{C}^{11/16}$ ), 134.3 (s,  $\text{C}^{11/16}$ ), 133.3 (s,  $\text{C}^8$ ), 128.4 (s,  $\text{C}^{18+19}$ ), 127.5 (s,  $\text{C}^5$ ), 127.2 (s,  $\text{C}^{13/14}$ ), 127.1 (s,  $\text{C}^{13/14}$ ), 126.0 (s,  $\text{C}^6$ ), 113.0 (s,  $\text{C}^{12/15}$ ), 112.8 (s,  $\text{C}^{12/15}$ ), 52.5 (s, CH<sub>2</sub>-N), 41.3 (s, HN-CH<sub>2</sub>-CH<sub>2</sub>), 36.7 (s, N-CH<sub>3</sub>), 35.7 (s,  $\text{C}(\text{CH}_3)_3$ ), 31.1 (s,  $\text{C}(\text{CH}_3)_3$ ).  $^{19}\text{F}\{^1\text{H}\}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 282.38 MHz, 293 K)  $\delta$  -73.2 (d,  $^1J_{\text{P-F}} = 711$  Hz, 6 F, PF<sub>6</sub>).

$[(\text{C}^{\text{N}}\text{P}^{\text{Z}}\text{N})\text{Au}(1\text{-methyl-3-(4-(4-aminobutyl)carboxamid)benzylbenzimidazol-2-ylidene})]\text{PF}_6$  (**AuC<sub>2</sub>NH<sub>2</sub>**)

Following the procedure described for **AuC<sub>2</sub>NH<sub>2</sub>**, the compound was made from **2** (200 mg, 0.17 mmol) and 1,4-diaminobutane (302 mg, 3.4 mmol) and obtained as an orange powder (155 mg, 0.15 mmol, 89 % yield). Anal. Calcd for  $\text{C}_{44}\text{H}_{50}\text{N}_6\text{OAU}\text{PF}_6$  (1020.9): C, 51.77; H, 4.94; N, 8.23. Found: C, 51.65; H, 4.86; N, 8.31. IR ( $\text{cm}^{-1}$ ):  $\nu(\text{N-H})$  3305 (very broad),  $\nu(\text{C}=\text{O})$  1646.  $^1\text{H}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 300.13 MHz):  $\delta$  8.97 (s, 2 H,  $\text{H}^2$ ), 7.78-7.70 (d + m,  $^3J_{\text{H-H}} = 9.0$  Hz, 6 H,  $\text{H}^5 + \text{H}^{13-16}$ ), 7.48 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 2 H,  $\text{H}^{19}$ ), 7.42 (dd,  $^3J_{\text{H-H}} = 9.0$  Hz,  $^4J_{\text{H-H}} = 3.0$  Hz, 2 H,  $\text{H}^6$ ), 7.22 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 2 H,  $\text{H}^{18}$ ), 7.12 (broad t, 1 H,

C(O)NH), 6.91 (d,  $^4J_{\text{H-H}} = 3.0$  Hz, 2 H, H<sup>8</sup>), 5.69 (s, 2 H, N-CH<sub>2</sub>), 4.14 (s, 3 H, N-CH<sub>3</sub>), 3.30 (m, 2 H, CH<sub>2</sub><sup>21</sup>), 2.70 (t,  $^3J_{\text{H-H}} = 6.0$  Hz, CH<sub>2</sub><sup>24</sup>), 1.60 (m, 4 H, CH<sub>2</sub><sup>22</sup> + NH<sub>2</sub>), 1.48 (m, 2 H, CH<sub>2</sub><sup>23</sup>), 1.19 (s, 18 H, <sup>1</sup>Bu). <sup>13</sup>C{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 75.48 MHz, 293 K):  $\delta$  166.0 (s, C=O), 165.3 (s, C<sup>9</sup>), 163.2 (s, C<sub>carbene</sub>), 158.4 (s, C<sup>7</sup>), 157.2 (s, C<sup>4</sup>), 144.8 (s, C<sup>3</sup>), 140.0 (s, C<sup>2</sup>), 136.6 (s, C<sup>20</sup>), 136.4 (s, C<sup>17</sup>), 134.9 (s, C<sup>11/16</sup>), 134.3 (s, C<sup>11/16</sup>), 133.3 (s, C<sup>8</sup>), 128.4 (s, C<sup>18/19</sup>), 128.3 (s, C<sup>18/19</sup>), 127.5 (s, C<sup>5</sup>), 127.2 (s, C<sup>13/14</sup>), 127.1 (s, C<sup>13/14</sup>), 126.1 (s, C<sup>6</sup>), 113.1 (s, C<sup>12/15</sup>), 112.9 (s, C<sup>12/15</sup>), 52.7 (s, CH<sub>2</sub>-N), 42.0 (s, CH<sub>2</sub><sup>21</sup> + <sup>24</sup>), 40.3 (s, CH<sub>2</sub><sup>22</sup>), 36.7 (s, N-CH<sub>3</sub>), 35.7 (s, C(CH<sub>3</sub>)<sub>3</sub>), 31.2 (s, C(CH<sub>3</sub>)<sub>3</sub>), 27.4 (s, CH<sub>2</sub><sup>23</sup>). <sup>19</sup>F{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 282.38 MHz, 293 K)  $\delta$  -73.2 (d,  $^1J_{\text{P-F}} = 711$  Hz, 6 F, PF<sub>6</sub>).

[(C<sup>AN</sup>Pz<sup>AN</sup>)Au(1-methyl-3-(4-(6-aminohexyl)carboxamid)benzylbenzimidazol-2-ylidene)]PF<sub>6</sub> (**AuC<sub>6</sub>NH<sub>2</sub>**)

Following the procedure described for **AuC<sub>2</sub>NH<sub>2</sub>**, the compound was made from **2** (153 mg, 0.13 mmol) and 1,6-diaminohexane (304 mg, 2.6 mmol) and obtained as an orange powder (122 mg, 0.12 mmol, 89 % yield). Anal. Calcd for C<sub>46</sub>H<sub>54</sub>N<sub>6</sub>O<sub>3</sub>SAuPF<sub>6</sub> (1048.9): C, 52.67; H, 5.19; N, 8.01. Found: C, 52.57; H, 5.31; N, 7.96. IR (cm<sup>-1</sup>):  $\nu$ (N-H) 3442,  $\nu$ (N-H) 3309,  $\nu$ (C=O) 1646. <sup>1</sup>H NMR (CD<sub>2</sub>Cl<sub>2</sub>, 300.13 MHz):  $\delta$  8.96 (s, 2 H, H<sup>2</sup>), 7.68-7.87 (m, 6 H, H<sup>5</sup> + CH<sup>12/15</sup>), 7.41-7.46 (m, 4 H, H<sup>6</sup> + H<sup>19</sup>), 7.24 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 2 H, H<sup>18</sup>), 6.90 (d,  $^4J_{\text{H-H}} = 3.0$  Hz, 2 H, H<sup>8</sup>), 6.02 (broad s, 1 H, C(O)NH), 5.69 (s, 2 H, N-CH<sub>2</sub>), 4.14 (s, 3 H, N-CH<sub>3</sub>), 3.29 (m, 2 H, CH<sub>2</sub><sup>21</sup>), 3.14 (t,  $^3J_{\text{H-H}} = 6.0$  Hz, 2 H, CH<sub>2</sub><sup>26</sup>), 1.50-1.57 (m, 4 H, CH<sub>2</sub><sup>22</sup> + NH<sub>2</sub>), 1.32-1.40 (m, 6 H, CH<sub>2</sub><sup>23-25</sup>) 1.19 (s, 18 H, <sup>1</sup>Bu). <sup>13</sup>C{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 75.48 MHz, 293 K):  $\delta$  166.2 (s, C=O), 165.2 (s, C<sup>9</sup>), 163.2 (s, C<sup>10</sup>), 158.3 (s, C<sup>7</sup>), 157.2 (s, C<sup>4</sup>), 144.8 (s, C<sup>3</sup>), 140.0 (s, C<sup>2</sup>), 136.8 (s, C<sup>20</sup>), 134.3 (s, C<sup>17</sup>), 134.2 (s, C<sup>11/16</sup>), 133.3 (s, C<sup>11/16</sup>), 128.5 (s, C<sup>8</sup>), 128.3 (s, C<sup>18/19</sup>), 128.2 (s, C<sup>18/19</sup>), 127.5 (s, C<sup>5</sup>), 127.2 (s, C<sup>13/14</sup>), 127.1 (s, C<sup>13/14</sup>), 126.0 (s, C<sup>6</sup>), 113.0 (s, C<sup>12/15</sup>), 112.9 (s, C<sup>12/15</sup>), 52.6 (s, CH<sub>2</sub>-N), 42.3 (s, CH<sub>2</sub><sup>26</sup>), 40.3 (s, CH<sub>2</sub><sup>21</sup>), 36.7 (s, N-CH<sub>3</sub>), 35.7 (s, C(CH<sub>3</sub>)<sub>3</sub>), 31.4 (s, CH<sub>2</sub><sup>22</sup>), 31.1 (s, C(CH<sub>3</sub>)<sub>3</sub>), 26.8 (s, CH<sub>2</sub><sup>23-25</sup>). <sup>19</sup>F{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 282.38 MHz, 293 K)  $\delta$  -73.2 (d,  $^1J_{\text{P-F}} = 709$  Hz, 6 F, PF<sub>6</sub>).

[(C<sup>AN</sup>Pz<sup>AN</sup>)Au(NHC-C<sub>2</sub>-biotin)]PF<sub>6</sub> (**AuC<sub>2</sub>Biot**)

**AuC<sub>2</sub>NH<sub>2</sub>** (40 mg, 0.040 mmol) and **Biotin-NHS** (14 mg, 0.040 mmol) were dissolved in dry DMF (0.5 mL). The reaction was stirred at room temperature overnight. Upon addition of diethyl ether an orange precipitate was formed, which was collected and recrystallized from a mixture dichloromethane/light petroleum to give the pure compound as an orange powder (49 mg, 0.039 mmol, 99 % yield). Anal. Calcd for C<sub>52</sub>H<sub>60</sub>N<sub>8</sub>O<sub>3</sub>SAuPF<sub>6</sub> (1219.1): C, 51.23; H, 4.96; N, 9.19. Found: C, 51.15; H, 4.98; N, 9.12. IR (cm<sup>-1</sup>):  $\nu$ (N-H) 3430,  $\nu$ (N-H) 3291,  $\nu$ (C=O) 1731,  $\nu$ (C=O) 1704,  $\nu$ (C=O) 1647. <sup>1</sup>H NMR (CD<sub>2</sub>Cl<sub>2</sub>, 300.13 MHz):  $\delta$  8.96 (s, 2 H, H<sup>2</sup>), 7.84-7.70 (m, 6 H, H<sup>5</sup> + H<sup>13/16</sup>), 7.53 (m + d,  $^3J_{\text{H-H}} = 9.0$  Hz, 3 H, H<sup>19</sup> + NH<sub>amide</sub>), 7.40 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 2 H, H<sup>6</sup>), 7.23 (d,  $^3J_{\text{H-H}} = 9.0$  Hz, 2 H, H<sup>18</sup>), 6.89 (s, 3 H, H<sup>8</sup> + NH<sub>amide</sub>), 5.97 (s, 1 H, NH<sub>urea</sub>), 5.72 (s, 2 H, N-CH<sub>2</sub>), 5.10 (s, 1 H, NH<sub>urea</sub>), 4.38 (s, 1 H, H<sup>29/30</sup>), 4.18 (s, 1 H, H<sup>29/30</sup>), 4.13 (s, 3 H, N-CH<sub>3</sub>), 3.36 (s, 4 H, CH<sub>2</sub><sup>21</sup> + <sup>22</sup>), 3.03 (s, 1 H, H<sup>27</sup>), 2.81 (m, 1 H, H<sup>28</sup>), 2.57 (d,  $^3J_{\text{H-H}} = 15$  Hz, H<sup>28</sup>), 2.12 (broad s, 2 H, CH<sub>2</sub><sup>23</sup>), 1.77 (m, 2 H, CH<sub>2</sub><sup>24/25/26</sup>), 1.52 (m, 2 H, CH<sub>2</sub><sup>24/25/26</sup>), 1.29 (m, 2 H, CH<sub>2</sub><sup>24/25/26</sup>), 1.18 (s, 18 H, <sup>1</sup>Bu). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>52</sub>H<sub>60</sub>N<sub>8</sub>O<sub>3</sub>SAu]<sup>+</sup> (1073.4169): measured *m/z* 1073.4166 [M-PF<sub>6</sub>]<sup>+</sup>, *positive mode exact mass* for [C<sub>52</sub>H<sub>60</sub>N<sub>8</sub>O<sub>3</sub>SAuNa]<sup>2+</sup> (1096.4072): measured *m/z* 548.2024 [M-PF<sub>6</sub>+Na]<sup>2+</sup>.

[(C<sup>AN</sup>Pz<sup>AN</sup>)Au(NHC-C<sub>4</sub>-biotin)]PF<sub>6</sub> (**AuC<sub>4</sub>Biot**)

Following the procedure described for **AuC<sub>2</sub>Biot**, the compound was made from **AuC<sub>4</sub>NH<sub>2</sub>** (40 mg, 0.039 mmol) and **Biotin-NHS** (14 mg, 0.039 mmol) and obtained as a yellow powder (48 mg, 0.038 mmol, 98 % yield). Anal. Calcd for C<sub>54</sub>H<sub>64</sub>N<sub>8</sub>O<sub>3</sub>SAuPF<sub>6</sub>·4H<sub>2</sub>O (1319.2): C, 49.17; H, 5.50; N, 8.49. Found: C, 49.44; H, 5.05; N, 9.65. IR (cm<sup>-1</sup>):  $\nu$ (N-H) 3429,  $\nu$ (N-H) 3290 (very broad),  $\nu$ (C=O) 1704,  $\nu$ (C=O) 1642. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300.13 MHz):  $\delta$  9.37 (s, 2 H, H<sup>2</sup>), 8.34 (broad s, 1 H, NH<sub>amide</sub>), 8.03-8.09 (m, 3 H, H<sup>13/14</sup> + H<sup>19</sup>), 7.84 (d,  $^3J_{\text{H-H}} = 7.8$  Hz, 1 H, H<sup>12/15</sup>), 7.75 (broad s, 1 H, NH<sub>amide</sub>), 7.59-7.69 (m, 4 H, H<sup>12/15</sup> + H<sup>13/14</sup> + H<sup>5</sup>), 7.35-7.43 (m, 4 H, H<sup>6</sup> + H<sup>18</sup>), 6.98 (s, 2 H, H<sup>8</sup>), 6.79 (s, 1 H, NH<sub>urea</sub>), 6.69 (s, 1 H, NH<sub>urea</sub>), 5.94 (s, 2 H, N-CH<sub>2</sub>), 4.43 (m, 1 H, H<sup>31/32</sup>), 4.30 (s, 1 H, H<sup>31/32</sup>), 4.17 (s, 3 H, N-CH<sub>3</sub>), 3.66 (broad m, 2 H, CH<sub>2</sub><sup>21</sup>), 3.0 (broad m, 2 H, H<sup>24</sup>), 2.89 (s, 1 H, H<sup>30</sup>), 2.73 (s, H<sup>30</sup>), 2.03 (m, 2 H, H<sup>25</sup>), 1.71 (broad s, 1 H, CH<sub>2</sub><sup>29</sup>), 1.35-1.51 (m, 10 H, CH<sub>2</sub><sup>22-23</sup> + CH<sub>2</sub><sup>26-28</sup>), 1.14 (s, 18 H, <sup>1</sup>Bu). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>54</sub>H<sub>64</sub>N<sub>8</sub>O<sub>3</sub>SAu]<sup>+</sup> (1101.4482): measured *m/z* 1101.4473 [M-PF<sub>6</sub>]<sup>+</sup>, *positive mode exact mass* for [C<sub>54</sub>H<sub>64</sub>N<sub>8</sub>O<sub>3</sub>SAuK]<sup>2+</sup> (1140.4125): measured *m/z* 570.2152 [M-PF<sub>6</sub>+K]<sup>2+</sup>.

[(C<sup>AN</sup>Pz<sup>AN</sup>)Au(NHC-C<sub>6</sub>-biotin)]PF<sub>6</sub> (**AuC<sub>6</sub>Biot**)

Following the procedure described for **AuC<sub>2</sub>Biot**, the compound was made from **AuC<sub>6</sub>NH<sub>2</sub>** (40 mg, 0.038 mmol) and **Biotin-NHS** (13 mg, 0.038 mmol) and obtained as a yellow powder (47 mg, 0.037 mmol, 97 % yield). Anal. Calcd for C<sub>56</sub>H<sub>68</sub>N<sub>8</sub>O<sub>3</sub>SAuPF<sub>6</sub>·2H<sub>2</sub>O (1329.3): C, 51.26; H, 5.61; N, 8.54. Found: C, 51.47; H, 5.94; N, 8.12. IR (cm<sup>-1</sup>):  $\nu$ (N-H) 3434,  $\nu$ (N-H) 3296,  $\nu$ (C=O) 1702,  $\nu$ (C=O) 1646. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300.13 MHz):  $\delta$  10.56 (broad s, 1 H, NH<sub>amide</sub>), 9.37 (s, 2 H, H<sup>2</sup>), 8.33 (broad s, 1 H, NH<sub>amide</sub>), 8.04-8.06 (m, 3 H, H<sup>13/14</sup> + H<sup>19</sup>), 7.84 (d,  $^3J_{\text{H-H}} = 7.8$  Hz, 1 H, H<sup>12/15</sup>), 7.59-7.74 (m, 4 H, H<sup>12/15</sup> + H<sup>13/14</sup> + H<sup>5</sup>), 7.35-7.38 (m, 4 H, H<sup>6</sup> + H<sup>18</sup>), 6.98 (s, 2 H, H<sup>8</sup>), 6.43 (s, 1 H, NH<sub>urea</sub>), 6.37 (s, 1 H, NH<sub>urea</sub>), 5.94 (s, 2 H, N-CH<sub>2</sub>), 4.29 (m, 1 H, H<sup>33/34</sup>), 4.17 (m, 4 H, N-CH<sub>3</sub> + H<sup>33/34</sup>), 3.14 (broad m, 2 H, CH<sub>2</sub><sup>21</sup>), 2.99 (broad m, 2 H, CH<sub>2</sub><sup>26</sup>), 2.81 (s, 2 H, H<sup>32</sup>), 2.03 (m, 2 H, H<sup>27</sup>), 1.63 (m, 1 H, CH<sub>3</sub><sup>31</sup>), 1.23-1.41 (m, 14 H, CH<sub>2</sub><sup>22-25</sup> + CH<sub>2</sub><sup>28-30</sup>), 1.14 (s, 18 H, <sup>1</sup>Bu). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>56</sub>H<sub>68</sub>N<sub>8</sub>O<sub>3</sub>SAu]<sup>+</sup> (1129.4795): measured *m/z* 1129.4784 [M-PF<sub>6</sub>]<sup>+</sup>, *positive mode exact mass* for [C<sub>56</sub>H<sub>68</sub>N<sub>8</sub>O<sub>3</sub>SAuNa]<sup>2+</sup> (1152.4698): measured *m/z* 576.2334 [M-PF<sub>6</sub>+Na]<sup>2+</sup>.

[(C<sup>AN</sup>Pz<sup>AN</sup>)Au(NHC-C<sub>2</sub>-ethynylestradiol)]PF<sub>6</sub> (**AuC<sub>2</sub>Est**)

**AuC<sub>2</sub>NH<sub>2</sub>** (32 mg, 0.032 mmol) and **ethynylestradiol-NHS** (17 mg, 0.032 mmol) were dissolved in dry acetonitrile (1 mL) and K<sub>2</sub>CO<sub>3</sub> (9 mg, 0.064 mmol, 2 eq.) was added. The mixture was stirred at room temperature overnight. The solvent was removed under vacuum and the residue dissolved in dichloromethane. After filtration, the dichloromethane solution was concentrated under vacuum. Upon addition of diethyl ether a pale yellow precipitate was formed which was collected and recrystallized from a mixture dichloromethane/light petroleum to give the pure compound as a yellow powder (34 mg, 0.025 mmol, 76 % yield). Anal. Calcd for C<sub>69</sub>H<sub>74</sub>N<sub>6</sub>O<sub>4</sub>AuPF<sub>6</sub>·2H<sub>2</sub>O (1427.3): C, 58.06; H, 5.37; N, 5.89. Found: C, 57.99; H, 5.28; N, 6.35. IR (cm<sup>-1</sup>):  $\nu$ (N-H) 3430,  $\nu$ (N-H + O-H) 3368 (very broad),  $\nu$ (C $\equiv$ C) not visible,  $\nu$ (C=O) 1708. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300.13 MHz):  $\delta$  9.36 (s, 2 H, H<sup>2</sup>), 9.02 (s, 1 H, OH<sub>phenol</sub>), 8.59 (s, 1 H, NH), 8.49 (s, 1 H, NH), 8.05 (s, 3 H, CH<sub>arom.</sub>), 7.79 (d,  $^3J_{\text{H-H}} = 7.8$  Hz, 2 H, CH<sub>arom.</sub>), 7.62 (m, 4 H, CH<sub>arom.</sub>), 7.37-7.47 (m, 5



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H, CH<sub>arom.</sub>), 7.06 (d, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 1 H, H<sup>21</sup>), 6.99 (s, 2 H, H<sup>8</sup>), 6.51 (d, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 1 H, H<sup>22</sup>), 6.44 (s, 1 H, H<sup>23</sup>), 5.94 (s, 2 H, N-CH<sub>2</sub>), 5.52 (s, 1 H, OH), 4.16 (s, 3 H, N-CH<sub>3</sub>), 2.89 (s, 2 H, CH<sub>2</sub><sub>steroid</sub>), 1.97-1.78 (m, 6 H, CH<sub>steroid</sub>), 1.23-1.32 (m, 7 H, CH<sub>steroid</sub>), 1.13 (s, 18 H, <sup>t</sup>Bu), 0.82 (s, 3 H, CH<sub>3</sub><sub>steroid</sub>). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>69</sub>H<sub>72</sub>N<sub>6</sub>O<sub>4</sub>Au]<sup>+</sup> (1245.5275): measured *m/z* 1245.5268 [M-PF<sub>6</sub>]<sup>+</sup>.

[(C<sup>AN</sup>PZ<sup>AN</sup>)Au(NHC-C<sub>4</sub>-ethynylestradiol)]PF<sub>6</sub> (**AuC<sub>4</sub>Est**)

Following the procedure described for **AuC<sub>2</sub>Estra**, the compound was made from **AuC<sub>4</sub>NH<sub>2</sub>** (40 mg, 0.039 mmol), **ethynylestradiol-NHS** (20 mg, 0.039 mmol) and K<sub>2</sub>CO<sub>3</sub> (11 mg, 0.078 mmol) and obtained as a yellow powder (37 mg, 0.026 mmol, 67 % yield). Anal. Calcd for C<sub>71</sub>H<sub>76</sub>N<sub>6</sub>O<sub>4</sub>AuPF<sub>6</sub>·8H<sub>2</sub>O (1563.5): C, 54.54; H, 5.93; N, 5.38. Found: C, 54.56; H, 4.50; N, 5.18. IR (cm<sup>-1</sup>): ν(N-H, O-H) 3430, ν(N-H, O-H) 3311, ν(C≡C) not visible, ν(C=O) 1646. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300.13 MHz): δ 9.36 (s, 2 H, H<sup>2</sup>), 9.00 (s, 1 H, OH<sub>phenol</sub>), 8.51 (s, 1 H, NH), 8.34 (s, 1 H, NH), 8.04 (s, 3 H, CH<sub>arom.</sub>), 7.83 (m, 3 H, CH<sub>arom.</sub>), 7.57-7.68 (m, 5 H, CH<sub>arom.</sub>), 7.47 (d, <sup>3</sup>J<sub>H-H</sub> = 7.8 Hz, 1 H, CH<sub>bzim</sub>), 7.37 (m, 4 H, CH<sub>arom.</sub>), 7.06 (d, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 1 H, H<sup>21</sup>), 6.97 (s, 2 H, H<sup>8</sup>), 6.50 (d, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 1 H, H<sup>22</sup>), 6.43 (s, 1 H, H<sup>23</sup>), 5.93 (s, 2 H, N-CH<sub>2</sub>), 5.52 (s, 1 H, OH), 4.15 (s, 3 H, N-CH<sub>3</sub>), 3.20 (m, 4 H, CH<sub>2</sub>-NH), 2.73 (s, 2 H, CH<sub>2</sub><sub>steroid</sub>), 1.67-2.27 (m, 8 H, CH<sub>steroid</sub> + CH<sub>2</sub>), 1.23-1.48 (m, 9 H, CH<sub>steroid</sub> + CH<sub>2</sub>), 1.12 (s, 18 H, <sup>t</sup>Bu), 0.82 (s, 3 H, CH<sub>3</sub><sub>steroid</sub>). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>71</sub>H<sub>76</sub>N<sub>6</sub>O<sub>4</sub>Au]<sup>+</sup> (1273.5588): measured *m/z* 1273.5575 [M-PF<sub>6</sub>]<sup>+</sup>.

[(C<sup>AN</sup>PZ<sup>AN</sup>)Au(NHC-C<sub>6</sub>-ethynylestradiol)]PF<sub>6</sub> (**AuC<sub>6</sub>Est**)

Following the procedure described for **AuC<sub>2</sub>Estra**, the compound was made from **AuC<sub>4</sub>NH<sub>2</sub>** (50 mg, 0.048 mmol), **ethynylestradiol-NHS** (25 mg, 0.048 mmol) and K<sub>2</sub>CO<sub>3</sub> (13 mg, 0.096 mmol) and obtained as a yellow powder (55 mg, 0.038 mmol, 79 % yield). Anal. Calcd for C<sub>73</sub>H<sub>80</sub>N<sub>6</sub>O<sub>4</sub>AuPF<sub>6</sub> (1447.4): C, 60.58; H, 5.57; N, 5.81. Found: C, 60.36; H, 5.65; N, 5.96. IR (cm<sup>-1</sup>): ν(N-H, O-H) 3432, ν(N-H, O-H) 3318, ν(C≡C) not visible, ν(C=O) 1646. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300.13 MHz): δ 9.35 (s, 2 H, H<sup>2</sup>), 9.01 (s, 1 H, OH<sub>phenol</sub>), 8.49 (s, 1 H, NH), 8.31 (s, 1 H, CH<sub>arom.</sub>), 8.04 (m, 3 H, CH<sub>arom.</sub>), 7.83 (m, 2 H, CH<sub>arom.</sub>), 7.37-7.68 (m, 10 H, CH<sub>arom.</sub>), 6.97 (s + m, 3 H, H<sup>8</sup> + H<sup>21</sup>), 6.51 (d, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 1 H, H<sup>22</sup>), 6.43 (s, 1 H, H<sup>23</sup>), 5.93 (s, 2 H, N-CH<sub>2</sub>), 5.55 (m, 2 H, OH + NH), 4.16 (s, 3 H, N-CH<sub>3</sub>), 3.15-3.23 (m, 4 H, CH<sub>2</sub>-NH), 2.85 (m, 2 H, CH<sub>2</sub><sub>steroid</sub>), 1.73-1.98 (m, 6 H, CH<sub>steroid</sub> + CH<sub>2</sub>), 1.22-1.41 (m, 14 H, CH<sub>steroid</sub> + CH<sub>2</sub>), 1.13 (s, 18 H, <sup>t</sup>Bu), 0.82 (s, 3 H, CH<sub>3</sub><sub>steroid</sub>). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>73</sub>H<sub>80</sub>N<sub>6</sub>O<sub>4</sub>Au]<sup>+</sup> (1301.5901): measured *m/z* 1301.5887 [M-PF<sub>6</sub>]<sup>+</sup>.

[(C<sup>AN</sup>PZ<sup>AN</sup>)Au(NHC-C<sub>2</sub>-NHC-AuCl)]PF<sub>6</sub> (**AuC<sub>2</sub>Au**)

**AuC<sub>2</sub>NH<sub>2</sub>** (45 mg, 0.045 mmol) and **{MeBzim(Bz-4-CO<sub>2</sub>(C<sub>6</sub>F<sub>5</sub>))}AuCl** (30 mg, 0.045 mmol, 1 eq.) were stirred in distilled acetonitrile (6 mL) at room temperature for 5 h in the dark. The solvent was evaporated and the yellow solid residue was dissolved in dichloromethane. The solution was washed with saturated aqueous potassium carbonate solution and then with water. The organic phase was dried over sodium sulfate. Upon addition of light petroleum a yellow precipitate was formed that was collected, washed twice with light petroleum and dried to afford the pure product as a yellow powder (21 mg, 0.014 mmol, 95 % yield). Anal. Calcd for C<sub>58</sub>H<sub>58</sub>N<sub>8</sub>O<sub>2</sub>Au<sub>2</sub>ClPF<sub>6</sub>·C<sub>6</sub>H<sub>14</sub>

(1473.5): C, 49.22; H, 4.78; N, 7.18. Found: C, 49.91; H, 4.29; N, 6.96. IR (cm<sup>-1</sup>): ν(N-H) 3321 (very broad), ν(C=O) 1651 (broad). <sup>1</sup>H NMR (CD<sub>2</sub>Cl<sub>2</sub>, 300.13 MHz): δ 8.88 (s, 2 H, H<sup>2</sup>), 7.82-7.70 (m, 8 H, CH<sub>arom.</sub>), 7.54-7.34 (m, 11 H, 10 CH<sub>arom.</sub> + NH), 7.27 (broad m, 1 H, NH), 7.21 (d, <sup>3</sup>J<sub>H-H</sub> = 9.0 Hz, CH<sub>arom.</sub>), 6.83 (d, <sup>4</sup>J<sub>H-H</sub> = 3.0 Hz, 2 H, H<sup>8</sup>), 5.76 (s, 2 H, CH<sub>2</sub><sup>29</sup>), 5.65 (s, 2 H, CH<sub>2</sub><sup>18</sup>), 4.11 (s, 3 H, CH<sub>3</sub><sup>17</sup>), 4.09 (s, 3 H, CH<sub>3</sub><sup>36</sup>), 3.53 (broad s, 4 H, CH<sub>2</sub><sup>23+24</sup>), 1.15 (s, 18 H, <sup>t</sup>Bu). <sup>13</sup>C{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 75.48 MHz, 293 K): δ 179.6 (s, C<sup>37</sup>), 168.0 (s, C=O), 167.2 (s, C=O), 165.1 (s, C<sup>9</sup>), 163.4 (s, C<sup>10</sup>), 158.2 (s, C<sup>7</sup>), 157.1 (s, C<sup>3</sup>), 144.8 (s, C<sup>4</sup>), 140.1 (s, C<sup>2</sup>), 139.0 (s, C<sup>28</sup>), 137.1 (s, C<sup>19</sup>), 135.5 (s, C<sup>22/25</sup>), 134.8 (s, C<sup>22/25</sup>), 134.7 (s, C<sup>16/35</sup>), 134.6 (s, C<sup>16/35</sup>), 134.3 (s, C<sup>11</sup>), 133.3 (s, C<sup>30</sup>), 133.2 (s, C<sup>8</sup>), 128.6 (s, CH<sub>arom.</sub>), 128.5 (s, CH<sub>arom.</sub>), 128.2 (s, CH<sub>arom.</sub>), 127.9 (s, CH<sub>arom.</sub>), 127.6 (s, C<sup>5</sup>), 127.2 (s, CH<sub>bzim</sub>), 127.0 (s, CH<sub>bzim</sub>), 126.0 (s, C<sup>6</sup>), 125.3 (s, CH<sub>bzim</sub>), 113.0 (s, CH<sub>bzim</sub>), 112.8 (s, CH<sub>bzim</sub>), 112.4 (s, CH<sub>bzim</sub>), 112.1 (s, CH<sub>bzim</sub>), 52.7 (s, CH<sub>2</sub><sup>29</sup>), 52.5 (s, CH<sub>2</sub><sup>18</sup>), 41.4 (s, CH<sub>2</sub><sup>23+24</sup>), 36.7 (s, CH<sub>3</sub><sup>17</sup>), 35.8 (s, CH<sub>3</sub><sup>36</sup>), 35.7 (s, C(CH<sub>3</sub>)<sub>3</sub>), 31.1 (s, C(CH<sub>3</sub>)<sub>3</sub>). <sup>19</sup>F{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 282.38 MHz, 293 K) δ -73.2 (d, <sup>1</sup>J<sub>P-F</sub> = 711 Hz, 6 F, PF<sub>6</sub>). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>58</sub>H<sub>58</sub>N<sub>8</sub>O<sub>2</sub>Au<sub>2</sub>Cl]<sup>+</sup> (1327.3697): measured *m/z* 1327.3688 [M-PF<sub>6</sub>]<sup>+</sup>, *positive mode exact mass* for [C<sub>58</sub>H<sub>58</sub>N<sub>8</sub>O<sub>2</sub>Au<sub>2</sub>]<sup>2+</sup> (1292.4014): measured *m/z* 646.1985 [M-Cl-PF<sub>6</sub>]<sup>2+</sup>.

[(C<sup>AN</sup>PZ<sup>AN</sup>)Au(NHC-C<sub>4</sub>-NHC-AuCl)]PF<sub>6</sub> (**AuC<sub>4</sub>Au**)

Following the procedure described for **AuC<sub>2</sub>Au**, the compound was made from **AuC<sub>4</sub>NH<sub>2</sub>** (34 mg, 0.033 mmol) and **{MeBzim(Bz-4-CO<sub>2</sub>(C<sub>6</sub>F<sub>5</sub>))}AuCl** (22 mg, 0.033 mmol) and obtained as a yellow powder (47 mg, 0.031 mmol, 95 % yield). Anal. Calcd for C<sub>60</sub>H<sub>62</sub>N<sub>8</sub>O<sub>2</sub>Au<sub>2</sub>ClPF<sub>6</sub>·7H<sub>2</sub>O (1627.7): C, 44.28; H, 4.71; N, 6.88. Found: C, 44.19; H, 3.57; N, 7.19. IR (cm<sup>-1</sup>): ν(N-H) 3330, ν(C=O) 1646. <sup>1</sup>H NMR (CD<sub>2</sub>Cl<sub>2</sub>, 300.13 MHz): δ 8.92 (s, 2 H, H<sup>2</sup>), 7.83-7.71 (m, 8 H, CH<sub>arom.</sub>), 7.51-7.37 (m, 10 H, 10 CH<sub>arom.</sub>), 7.22 (d, <sup>3</sup>J<sub>H-H</sub> = 9.0 Hz, CH<sub>arom.</sub>), 6.83 (d, <sup>4</sup>J<sub>H-H</sub> = 3.0 Hz, 2 H, H<sup>8</sup>), 6.75 (broad m, 1 H, NH), 6.56 (broad m, 1 H, NH), 5.74 (s, 2 H, CH<sub>2</sub><sup>29</sup>), 5.67 (s, 2 H, CH<sub>2</sub><sup>18</sup>), 4.12 (s, 3 H, CH<sub>3</sub><sup>17</sup>), 4.07 (s, 3 H, CH<sub>3</sub><sup>36</sup>), 3.32-3.41 (m, 4 H, CH<sub>2</sub><sup>23+26</sup>), 1.59 (broad s, 4 H, CH<sub>2</sub><sup>24+25</sup>), 1.16 (s, 18 H, <sup>t</sup>Bu). <sup>13</sup>C{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 75.48 MHz, 293 K): δ 179.6 (s, C<sup>39</sup>), 167.2 (s, C=O), 166.3 (s, C=O), 165.1 (s, C<sup>9</sup>), 163.3 (s, C<sup>10</sup>), 158.2 (s, C<sup>7</sup>), 157.2 (s, C<sup>3</sup>), 144.8 (s, C<sup>4</sup>), 140.1 (s, C<sup>2</sup>), 138.8 (s, C<sup>30</sup>), 136.9 (s, C<sup>19</sup>), 136.1 (s, C<sup>22/27</sup>), 135.3 (s, C<sup>22/27</sup>), 134.8 (s, C<sup>16/37</sup>), 134.6 (s, C<sup>16/36</sup>), 134.3 (s, C<sup>11</sup>), 133.3 (s, C<sup>32</sup>), 133.2 (s, C<sup>8</sup>), 128.6 (s, CH<sub>arom.</sub>), 128.4 (s, CH<sub>arom.</sub>), 128.2 (s, CH<sub>arom.</sub>), 127.9 (s, CH<sub>arom.</sub>), 127.6 (s, C<sup>5</sup>), 127.2 (s, CH<sub>bzim</sub>), 127.1 (s, CH<sub>bzim</sub>), 126.0 (s, C<sup>6</sup>), 125.3 (s, CH<sub>bzim</sub>), 113.0 (s, CH<sub>bzim</sub>), 112.8 (s, CH<sub>bzim</sub>), 112.4 (s, CH<sub>bzim</sub>), 112.1 (s, CH<sub>bzim</sub>), 52.7 (s, CH<sub>2</sub><sup>31</sup>), 52.5 (s, CH<sub>2</sub><sup>18</sup>), 39.9 (s, CH<sub>2</sub><sup>23+26</sup>), 36.7 (s, CH<sub>3</sub><sup>17</sup>), 35.8 (s, CH<sub>3</sub><sup>38</sup>), 35.7 (s, C(CH<sub>3</sub>)<sub>3</sub>), 31.2 (s, C(CH<sub>3</sub>)<sub>3</sub>), 27.3 (s, CH<sub>2</sub><sup>24/25</sup>), 26.9 (s, CH<sub>2</sub><sup>24/25</sup>). <sup>19</sup>F{<sup>1</sup>H} NMR (CD<sub>2</sub>Cl<sub>2</sub>, 282.38 MHz, 293 K) δ -73.2 (d, <sup>1</sup>J<sub>P-F</sub> = 711 Hz, 6 F, PF<sub>6</sub>). ESI-MS (MeOH), *positive mode exact mass* for [C<sub>60</sub>H<sub>62</sub>N<sub>8</sub>O<sub>2</sub>Au<sub>2</sub>Cl]<sup>+</sup> (1355.4010): measured *m/z* 1327.3688 [M-PF<sub>6</sub>]<sup>+</sup>, *positive mode exact mass* for [C<sub>60</sub>H<sub>62</sub>N<sub>8</sub>O<sub>2</sub>Au<sub>2</sub>]<sup>2+</sup> (1320.4327): measured *m/z* 660.2144 [M-Cl-PF<sub>6</sub>]<sup>2+</sup>.

[(C<sup>AN</sup>PZ<sup>AN</sup>)Au(NHC-C<sub>6</sub>-NHC-AuCl)]PF<sub>6</sub> (**AuC<sub>6</sub>Au**)

Following the procedure described for **AuC<sub>2</sub>Au**, the compound was made from **AuC<sub>6</sub>NH<sub>2</sub>** (50 mg, 0.048 mmol, 1 eq.) and **{MeBzim(Bz-4-CO<sub>2</sub>(C<sub>6</sub>F<sub>5</sub>))}AuCl** (32 mg, 0.048 mmol, 1 eq.) and obtained as a yellow powder (47 mg, 0.031 mmol, 95 % yield). Anal. Calcd for

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$C_{62}H_{66}N_8O_2Au_2ClPF_6$  (1529.6): C, 48.68; H, 4.35; N, 7.33. Found: C, 48.80; H, 4.18; N, 7.18. IR ( $cm^{-1}$ ):  $\nu(N-H)$  3434,  $\nu(N-H)$  3330,  $\nu(C=O)$  1645.  $^1H$  NMR ( $CD_2Cl_2$ , 300.13 MHz):  $\delta$  8.95 (s, 2 H,  $H^2$ ), 7.83-7.68 (m, 9 H,  $CH_{arom.}$ ), 7.51-7.35 (m, 9 H,  $CH_{arom.}$ ), 7.24 (d,  $^3J_{H-H} = 9.0$  Hz, 2 H,  $CH_{arom.}$ ), 6.89 (s, 2 H,  $H^8$ ), 6.52 (broad m, 1 H, NH), 6.36 (broad m, 1 H, NH), 5.73 (s, 2 H,  $CH_2^{29}$ ), 5.70 (s, 2 H,  $CH_2^{18}$ ), 4.13 (s, 3 H,  $CH_3^{17}$ ), 4.07 (s, 3 H,  $CH_3^{36}$ ), 3.27-3.38 (m, 4 H,  $CH_2^{23+28}$ ), 1.36-1.59 (m, 8 H,  $CH_2^{24-27}$ ), 1.17 (s, 18 H,  $tBu$ ).  $^{13}C\{^1H\}$  NMR ( $CD_2Cl_2$ , 75.48 MHz, 293 K):  $\delta$  179.2 (s,  $C^{41}$ ), 167.0 (s,  $C=O$ ), 166.3 (s,  $C=O$ ), 165.1 (s,  $C^9$ ), 163.1 (s,  $C^{10}$ ), 158.1 (s,  $C^7$ ), 157.1 (s,  $C^3$ ), 144.8 (s,  $C^4$ ), 139.9 (s,  $C^2$ ), 138.5 (s,  $C^{32}$ ), 136.9 (s,  $C^{19}$ ), 136.0 (s,  $C^{22/29}$ ), 135.2 (s,  $C^{22/29}$ ), 134.8 (s,  $C^{16/39}$ ), 134.5 (s,  $C^{16/39}$ ), 134.2 (s,  $C^{11}$ ), 133.2 (s,  $C^8$ ), 131.8 (s,  $C^{35}$ ), 128.4 (s,  $CH_{arom.}$ ), 128.3 (s,  $CH_{arom.}$ ), 128.0 (s,  $CH_{arom.}$ ), 127.7 (s,  $CH_{arom.}$ ), 127.5 (s,  $C^5$ ), 127.1 (s,  $CH_{bzim.}$ ), 127.0 (s,  $CH_{bzim.}$ ), 125.9 (s,  $C^6$ ), 125.3 (s,  $CH_{bzim.}$ ), 113.0 (s,  $CH_{bzim.}$ ), 112.8 (s,  $CH_{bzim.}$ ), 112.3 (s,  $CH_{bzim.}$ ), 112.0 (s,  $CH_{bzim.}$ ), 52.5 (s,  $CH_2^{18+33}$ ), 39.8 (s,  $CH_2^{23/28}$ ), 39.6 (s,  $CH_2^{23/28}$ ), 36.6 (s,  $CH_3^{17/40}$ ), 35.6 (s,  $C(CH_3)_3$ ), 31.1 (s,  $C(CH_3)_3$ ), 29.7 (s,  $CH_2^{24/27}$ ), 29.6 (s,  $CH_2^{24/27}$ ), 26.3 (s,  $CH_2^{25/26}$ ), 26.1 (s,  $CH_2^{25/26}$ ).  $^{19}F\{^1H\}$  NMR ( $CD_2Cl_2$ , 282.38 MHz, 293 K)  $\delta$  -73.2 (d,  $^1J_{P-F} = 711$  Hz, 6 F,  $PF_6$ ). ESI-MS (MeOH), *positive mode exact mass* for  $[C_{62}H_{66}N_8O_2Au_2Cl]^+$  (1383.4323): measured  $m/z$  1383.4331  $[M-PF_6]^+$ , *positive mode exact mass* for  $[C_{62}H_{66}N_8O_2Au_2]^{2+}$  (1348.4640): measured  $m/z$  674.2313  $[M-Cl-PF_6]^{2+}$ .

## Proliferation assay

Human A549 cancer cell lines (from ECACC) were cultured in RPMI 1640 medium with 10% fetal calf serum, 2mM L-glutamine, 100U/mL penicillin and 100  $\mu$ g/mL streptomycin (Invitrogen). Cells were maintained in a humidified atmosphere at 37 °C and 5%  $CO_2$ . The human MCF-7, MDA-MB-231, HCT116 cancer cell lines, human embryonic kidney HEK-293 cells and the human MRC-5 fibroblasts (from ECACC) were cultured in DMEM medium with 10% fetal calf serum, 2mM L-glutamine, 100U/mL penicillin and 100  $\mu$ g/mL streptomycin (Invitrogen). Cells were maintained in a humidified atmosphere at 37 °C and 5%  $CO_2$ . Inhibition of cancer cell proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium (MTS) assay using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) and following the manufacturer's instructions. Briefly, cells ( $8 \times 10^3/100$   $\mu$ L for A549, MCF-7, MDA-MB-231, HCT116 and HEK-293,  $2 \times 10^3/100$   $\mu$ L for MRC-5) were seeded in 96-well plates and left untreated or treated with 1  $\mu$ L of DMSO (vehicle control) or 1  $\mu$ L of complexes diluted in DMSO at different concentrations in triplicate for 72 h at 37 °C with 5%  $CO_2$ . Following this, MTS assay reagent was added for 4 h and absorbance measured at 490 nm using a Polarstar Optima microplate reader (BMG Labtech).  $IC_{50}$  values were calculated using GraphPad Prism Version 5.0 software.

## Uptake study

MCF-7, HCT-116 and MDA-MB-231 cells were grown in 75  $cm^2$  flasks up to 70 % of confluence in 10 mL of culture medium. Compounds **AuC<sub>6</sub>Biot**, **AuC<sub>6</sub>Estra**, **AuC<sub>6</sub>Au** and **AuNHC** as control were added to the flasks (100  $\mu$ L of 1 mM solution in DMSO) and incubated for 6 h at 37 °C with 5%  $CO_2$ . Negative controls were used by incubating cells with DMSO alone in the same conditions. After

removal of the medium and washing of the cells with PBS pH 7.4, the cells were detached using a trypsin solution. After quenching of trypsin with fresh medium, centrifugation and removal of the supernatant, the cell pellet was resuspended into 1 mL of PBS pH 7.4 and split into twice 500  $\mu$ L for metal and protein quantification. The number of cells (expressed in million cells) of each sample was determined by measuring the protein content of the treated samples using a BCA assay (ThermoFischer Scientific) corrected by the amount of protein/ $10^6$  cells determined for each cell type by measuring the protein content of an untreated sample and divided by the corresponding number of cells measured with a hemacytometer. Microwave digestion was used to solvate the samples to liquid form. Nitric acid and hydrogen peroxide were used in a Milestone Ethos 1 microwave system using SK-10 10 place carousel. The digest was ramped to 200 °C in 15 min holding at 200 °C for 15 min. Sample was weighed into a microwave vessel before digestion, and decanted and rinsed into a pre weighed PFA bottle after digestion. ICP-MS samples were spiked with rhodium internal standard and run on a Thermo X series 1 ICP-MS. Isotopes selected were  $^{63}Cu$ ,  $^{65}Cu$ ,  $^{107}Ag$ ,  $^{109}Ag$  and  $^{197}Au$ . Certified standards and independent reference were used for accuracy. Acid blanks were run through the system and subtracted from sample measurements before corrections for dilution.

## FRET assay

The initial FRET melting screen was performed using a fluorescence resonance energy transfer (FRET) DNA melting based assay. The sequences used was hTeloG<sub>FRET</sub> (5'-FAM-d[GGG-TTA-GGG-TTA-GGG-TTA-GGG]-TAMRA-3'). The labelled oligonucleotides (donor fluorophore FAM is 6-carboxyfluorescein; acceptor fluorophore TAMRA is 6-carboxytetramethyl-rhodamine) were prepared as a 220 nM solution in 10 mM sodium cacodylate buffer at pH 7.2 with 100 mM sodium chloride and then thermally annealed. Strip-tubes (QIAGEN) were prepared by aliquoting 20  $\mu$ L of the annealed DNA, followed by 0.2  $\mu$ L of the compound solution at a concentration of 100  $\mu$ M in DMSO. Control samples for each run were prepared with the same quantity of DMSO with the DNA in buffer. Fluorescence melt curves were determined in a QIAGEN Rotor-Gene Q-series PCR machine, using a total reaction volume of 20  $\mu$ L. Measurements were made with excitation at 470 nm and detection at 510 nm. Final analysis of the data was carried out using QIAGEN Rotor-Gene Q-series software and Origin or Excel.

## Acknowledgements

This work was supported by the European Research Council. M.B. is an ERC Advanced Investigator Award holder (grant no. 338944-GOCAT). The authors thank Elise Wright for her help with the DNA FRET-melting experiments and Graham Chilvers (UEA) for performing the ICP-MS measurements.

## Conflict of interest

The authors declare no conflict of interest.

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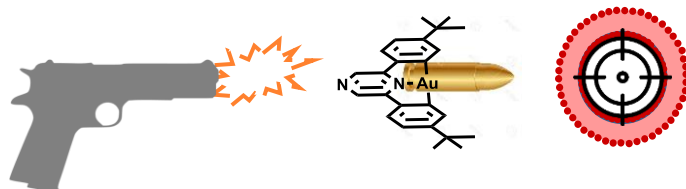
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**Gold(III) bioconjugates:** Modification of pincer-stabilized Au(III) NHC complexes gives bioconjugates and bimetallic complexes by a synthetically flexible method which enables the facile variation of the linker length and biovectors as well as the introduction of a second metal fragment. The compounds outline a possible strategy for improving selectivity in anti-cancer compounds.

**A gold(III) pincer ligand scaffold for the synthesis of binuclear and bioconjugated complexes: synthesis and anticancer potential**